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Rhythmic Response of Rhizome Growth to Light-break in Lotus (Nelumbo nucifera)

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We examined the response of rhizome growth to red light-break under different short daylengths in lotus (*Nelumbo nucifera*) seedlings. Maximum inhibitory response of rhizome enlargement to light-break under 10, 8 and 4 hr daylengths occurred 10, 8–10 and 12–14 hrs after the beginning of the dark period, respectively. It was found that rhythmic response to light-break is involved in rhizome growth of lotus.

INTRODUCTION

It is commonly recognized that modified parts of stem as tuber, corm and rhizome in geophytes, being used for asexual propagation, are the dormant organs for survival strategy of the plants during unfavorable period for their growth. The mechanisms of the formation of the dormant organs in these geophytes have been poorly understood. It has been known that tuber formation in *Solanum tuberosum* (Ewing and Struik, 1992; Snyder and Ewing, 1989), *Begonia evansiana* (Esashi and Nagao, 1958) and *Helianthus tuberosus* (Hamner and Long, 1939) is dependent on photoperiod. There are, however, no such reports in cormous and rhizomous plants in which the formation of dormant organs depend on environmental factors.

Lotus (Nelumbo nucifera) is an aquatic rhizomous plant. As temperature rises in the early spring, a new rhizome begins to elongate in a single direction with a few floating leaves from the enlarged rhizomes of the previous year. The rhizomes elongate and branch with many upright leaves, and rhizome girth and length become large and short in late summer, respectively. Finally, the rhizomes elongate up to about 11 m in main stem length and enlarge in three to four distal internodes to survive from incoming winter. Large field (more than 2 m²/plant) is resultantly required per plant for cultivation. Detailed investigation of the rhizome growth in underground consumes time and labor. Because of the reasons as mentioned above, the dynamics of rhizome transition to storage organ (physiological research) have been poorly understood in this species.

It was reported that photoperiodism involves a rhythmic change of the response of flowering and tuberization to light. Circadian rhythms in the response to a light-break in flowering have been well demonstrated in some other plants including SDPs as Perilla (Carr, 1952), Chenopodium rubrum (Cumming et al., 1965), Xanthium (Moore et al., 1967) and Pharbitis (Takimoto and Hamner, 1964) and LDPs as Hyoscyamus niger (Hsu and Hamner, 1967) and Lolium temulentum (Perilleux et al., 1994). Esashi (1963) reported that circadian rhythms of light-break in the formation of aerial tubers, which were induced by short daylength, in Begonia evansiana were also observed.

We report here the circadian rhythms to light-break under short daylength of rhizome enlargement in lotus.

MATERIALS AND METHODS

Plant Materials

Open pollinated seeds of N. nucifera 'Chugoku' were used in all experiments. The seeds were prepared for germination by soaking in conc. H_2SO_4 for $3\,hrs$ and rinsed with distilled water. They were then soaked in distilled water for one day at $25\,^{\circ}C$. After removing softened seed coats, the seeds were incubated in distilled water at $25\,^{\circ}C$ under continuous fluorescent light (approximately $40\,\mu\rm mol$ m $^{-2}\rm s^{-1}$) until germination (nine

We previously demonstrated that in lotus short daylength is a definite requirement for rhizome transition to storage organ (Masuda et al., 2006) by using seedlings. Giving light break in the middle of dark period under short daylength prevented from rhizome increase in girth, and yellow and red lights are the most effective than other light sources (Masuda et al., submitted). These are the similar responses of flowering in an SDP (short day plant) Xanthium strumarium, SDP Glycine max (Parker et al., 1946), LDP (long day plant) Hordeum vulgare (Borthwick et al., 1948) and LDP Hyoscyamus niger (Parker et al., 1950), and of tuberization in a few tuberous plants, Solanum tuberosum (Machackova et al., 1998) and Begonia evansiana (Esashi and Nagao, 1958; Esashi, 1966).

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days). Five seedlings for one treatment were transplanted into sandy soil containing 48g slow–release fertilizer (N : P : K = 10 : 10 : 10%) per plastic container ($45 \times 32 \times 23.5$ cm). The containers were filled with water, and the water was replaced weekly.

Rhizome enlargement index (= maximum internode diameter/internode length) was calculated as a parameter of rhizome growth in each internode after cultivation. The maximum rhizome enlargement index among all the internodes observed in each plant was used for calculating the average values. Starch grains accumulation in the cells of the youngest rhizomes was observed under a light microscope.

Light-break treatments

Nine-days old seedlings were transplanted and grown for 2 weeks under 14hr daylength with 8hr natural light (8:00-16:00) and 6 hr supplemental white light at 30 °C in a phytotron glass room of the Biotron Institute, Kyushu University. They were subsequently cultivated for 2 weeks under 10 (8 hr natural light (8:00–16:00) and 2 hr supplemental white light), 8 (8 hr natural light (8:00-16:00)) or 4 hr (4 hr natural light (10:00-14:00)) daylength. A single light-break for 5 minutes by red light was given after 2, 4, 6, 8, 10, 12, 14, 16 or 18 hrs from the beginning of the dark period. A white fluorescent tube (Mitsubishi FL20SSW/18) was used for white light. The red light was given from a red fluorescent tube (National FL20SR) filtered through a 3-mm thick red acrylic plate (Acrylite #102; Mitsubishi Rayon Co Ltd., Tokyo).

RESULTS

Three types of rhizome growth were obtained; low values (<0.2) of maximum rhizome enlargement index with or without accumulation of starch grains and the high values (>0.2) with starch grains accumulation in the cells of the youngest rhizome (Fig. 1). Maximum responsiveness to red light—break was, therefore, determined by the index and percentage of the plants with starch grains accumulation.

The plants grown under 10 hr daylength with 5 min red light-break after 2 hrs of the beginning of darkness showed high value of 0.66 in maximum rhizome enlargement index and starch grains accumulation was observed in all the plants (100%) similarly to those without light break (Fig. 2A). The later the light-break treatment from the beginning of the dark period was given, the lower the maximum rhizome enlargement index values were until the treatments after 10 hrs of darkness. More than a half of the plants accumulated starch grains in their rhizome cells whenever the light-break treatment was given except for that given at 10 hrs after the beginning of the dark period. The lowest value (0.18) of the index was observed with light-break given after 10 hrs of darkness, and the cells of the youngest rhizomes contained no starch grains in all the plants (0%).

Neither rhizome enlargement nor starch grains

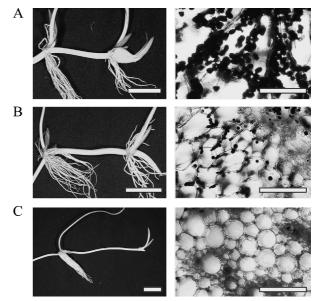


Fig. 1. Three types of rhizomes observed in maximum rhizome enlargement index and starch grains accumulation in the cells of the youngest rhizome. A; maximum rhizome enlargement index > 0.2 with starch grains accumulation, B; maximum rhizome enlargement index < 0.2 with starch grains accumulation and C; maximum rhizome enlargement index < 0.2 with no starch grains accumulation. Bars indicate 2.5 cm for left row and 100 μm for right row.</p>

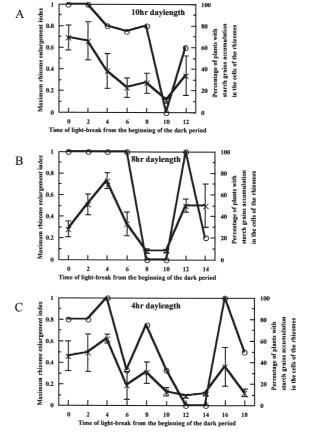


Fig. 2. Effect of time of light-break under different daylengths on maximum rhizome enlargement index of main rhizomes and percentage of the plants with starch grains accumulation in the cells of the youngest rhizome grown under 10 (A), 8 (B) and 4hr (C) daylengths. *; average of maximum rhizome enlargement index, ; percentage of the plants with starch grains accumulation. Vertical bars represent ± s.e.

accumulation was observed in the plants when the light–break treatment was given 8 and 10 hrs after the beginning of the dark period under 8hr daylength (Fig. 2B). The light–break treatments given at other times brought rhizome enlargement (>0.2) and/or starch grains accumulation.

No starch grains accumulation with maximum rhizome enlargement index of less than 0.2 was found only in the plants with the light-break treatments given 12 and 14 hrs after the beginning of the dark period (Fig. 2C). Although maximum rhizome enlargement indices of less than 0.2 were also found in the plants with the light-break treatments after 6, 10 and 18 hrs of darkness, more than 30% of the plants accumulated starch grains in their cells of the rhizomes.

DISCUSSION

It was found in the present study that the most effective times of light-break varied with daylength in lotus. A red light of one minute as a light-break was, however, most inhibitory at approximately the 8th hr of the dark periods irrespective of daylength (12, 8 and 4hr daylength) in aerial tuber formation of Begonia evansiana (Esashi, 1963). Lumsden et al. (1982) found that in dark-grown seedlings of *Pharbitis nil* exposed to a single photoperiod of less than about 6 hrs the first maximum inhibitory response of flowering to a light-break always occurred at approximately 15 hrs from the beginning of the light period. When the dark-grown seedlings were exposed to a single photoperiod longer than 6 hrs, it was always at 9 hrs after the end of the light period. It has been interpreted that the dark-grown seedlings initiate circadian rhythm in which the most effective times of light-break is at about 15 hrs from light period by light-on signal. The rhythm to light-break is, however, suspended and maintained when the dark-grown seedlings were exposed to a single light longer than 6 hrs, and the suspension is released by the transition from the light to dark. The released rhythm reaches its maximum responsiveness about 9 hrs after the beginning This interpretation from the results of Pharbitis nil is quite similar to those of Xanthium strumarium (Papenfuss and Salisbury, 1967). Maximum responsiveness of inhibition of rhizome enlargement in lotus to light-break under 10 and 8 hr daylengths occurred 10 and 8–10 hrs after the beginning of the dark period, respectively, whereas that under 4 hr daylength occurred 16-18 hrs after the beginning of the light period. The interpretation for Pharbitis nil and Xanthium strumarium seems to be applied to present results in lotus.

The plant must perceive light to act such a rhythmic response to light–break and it has been well recognized that phytochrome is involved in light perception. It was recently reported that phytochrome B mediated the light break–delayed flowering in $Oryza\ sativa$ (Ishikawa et al., 2005). Light exposure for 10 min in the middle of a 14 hr night (10 hr daylength) strongly suppressed the mRNA expression of Hd3a, a positive regulator of the

flowering, in the wild type, but the phyB mutation abolished the light break effect. The phyA and phyC mutation, however, had no effect on the expression of Hd3a mRNA. Phytochrome B may play an important role in the perception of the light break, and it may be also involved in the rhythmic response to light—break in lower

It is concluded that rhythmic response is involved in the responsiveness to light–break in rhizome enlargement of lotus.

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